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DEVELOPMENTAL OSSIFICATION SEQUENCE IN THE DISTAL THORACIC FLIPPER AS AN AID TO AGE DETERMINATION IN THE CALIFORNIA SEA LION, *Zalophus californianus*

By

A. G. Watson, J. S. Stewart, and T. M. Ganey

ADMINISTRATIVE REPORT LJ-86-22C

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Introduction and Literature Review

Determination of age is an essential prerequisite for the understanding of many dynamic aspects of mammalian life. It is also a vital life history parameter necessary for appropriate conservation and management of mammalian populations. The varied methods of determining age in mammals have been reviewed (Madsen, 1967; Morris, 1972). Among the more useful of these methods are those that analyze the periodic growth layers formed in teeth and bones since these layers indicate chronological, rather than physiological age (Klevezal' and Kleinenberg, 1969; Morris, 1978). These skeletal methods have been widely used in different groups of mammals.

Within the three major groups of marine mammals similar age determination methods have been used (Jonsgard, 1969). Tooth-age determination has become a fairly standard procedure in stock assessment and in population management of odontocete cetaceans (Scheffer and Myrick, 1980), and has also been applied to sirenians (Marsh, 1980; Myrick, 1985). For seals, tooth-age determination is a routine procedure in studies of these populations (Scheffer and Myrick, 1980). From the historical perspective we should recall that most of the pioneering work that demonstrated the value of growth layers in teeth as an indicator of age in mammals was, in fact, carried out in seals (Scheffer, 1950; Laws, 1952; but see also: reviews by Laws, 1962; and Scheffer and Myrick, 1980).

Specifically in California sea lions Zalophus californianus, the tooth-age determination technique has been used to analyze the age

structure in mature populations (Orr et al., 1970). Moreover, the reliable assessment of annual pup production is a vital determinant of populations dynamics and population size. Within Zalophus populations however, premature pupping with subsequent high pup mortality has been suspected along with an apparent increase in the premature pupping rate (Ronald et al., 1982; King, 1983). In field studies the carcases from dead premature pups may be confused with the carcases from dead full term and neonatal pups.

An age determination technique that would discriminate between these two groups of dead pups would contribute to more accurate assessment of annual pup production. The tooth-age determination methods, however, have not been useful to age neonatal pups (Beloborodov, 1975). What is required is a technique that would discriminate between late gestational prematurely born or aborted pups and early postnatal full term pups. During late gestation and the first year of life, mammals, and especially seals, characteristically show a pronounced and rapid growth in body size (Vaughan, 1972). Concomitantly, there is a marked growth in size and form of the skeleton. As the skeleton grows, new ossification centers and bony epiphyseal centers develop.

Radiographic techniques have been successfully used to study the development and maturation of the skeleton in man and domesticated mammals. As a result, reference standards for the correlation of skeletal development (based on radiograhic examination) and chronological age are now available for man (Greulich and Pyle, 1959; Warwick and Williams, 1973), dog (Hare, 1960; 1961; Andersen, 1970), cat (Smith, 1968; 1969; Horvath, 1983), and other domestic mammals (Getty, 1975.

In wild mammals, radiographic investigations of skeltal development are uncommon. Among the pioneers in this field are Washburn (1943) and Thomsen and Mortensen (1946). Studies that have since been reported have usually concentrated on the distal limb: for example, in the European hedgehog Erinaceus (Morris, 1971); in the raccoon Procyon (Petrides, 1959), in the red and gray foxes (Sullivan and Haugen, 1956) and in the black bear Ursus (Marks and Erickson, 1966). The use of radiographic assessment of skeletal development in marine mammals is more recent and more recent and relatively underdeveloped. Preliminary studies have been made in manatees Trichechus (Ribeino and Best, 1984; Watson and Hensen, 1985), and several reports are available for cetaceans (Hui, 1973; 1979; Lee, 1978; Lee and Ogden, 1982; Ogden et al., 1981).

Radiographic studies of seal flippers are also limited. Radiographs of single flippers have been published for the Ross seal Ommatophoca (King, 1965; 1983), the Weddell seal Leptonychotes (Pierard, 1971), the harp seal Phoca (Geraci, 1971), and the grey seal Halichoerus (King, 1983). Radiographs of malformed seal flippers have also been published (Marks and Burns, 1966; Tarasoff and Pierard, 1970). In addition, the radiographic appearance of thoracic and pelvic flipper bones of the harp seal has been briefly reported (Sumner-Smith, et al., 1972). This study examined four seal pups during the first year of life and two older seals, one at 2½ years and the other at 24 years of age. We are not aware of a study of seal flipper development during the neonatal period. A single radiograph of a thoracic flipper form a sub-adult California sea lion Zalophus has been published (King, 1983).

The objective of this report is to present the results of our radiographic study on the skeletal development in the distal part of the thoracic flipper from a series of California sea lions, Zalophus

californianus, most of which were pups from the neonatal period. The preliminary results of this study have been presented (Watson and Stewart, 1984).

Specimens

Fifty-one thoracic flippers from 39 California sea lions, Zalophus californianus, were studied. These flippers were removed from carcases of immature sea lions found dead on the beaches at the Z. californianus breeding rookeries on the California Channel Islands. The majority of flippers were collected during the spring (April-May) of 1982 and 1983 from either San Clemente or San Nicolas Islands. In the majority of the cases right flippers were collected and in 13 cases both right and left flippers were collected. Complete carcases were collected for three neonatal specimens (ZC-026, CWO-0873, CBH-1). Flippers from three pups-of-the-year and one second-year-pup were also collected. The largest flipper in this study was from a freshly necropsied 24-month-old female Z. californianus (82-M2), which was born and raised in a local seaquariaum. The collection dates, sites, specimen numbers, sex, and body measurement data are listed in Table 1.

Methods

Flippers were disarticulated at the elbow joint, removed from the carcase and frozen. Standard plain film radiographs were then made of each flipper with a soft X-ray technique in a Faxitron cabinet unit (Hewlett-Packard model number 43855A, 0.5 mm focal spot). The film-to-source distance was 61 cm and exposures were 42 kVp at 2 mA for 25 seconds on Kodak X-Omat TL-2 sheet film. All film was developed in a Kodak 90 second automatic X-Omat processor.

Radiographs were examined on a standard radiograph view box and the presence or absence of the various ossification centers was determined by the naked eye. Outline sketches of the bones in the flippers were drawn by making a tracing of the bones from selected representative radiographs.

Results

An examination of the radiographs in the 51 thoracic flippers has revealed varying numbers of carpal and epiphyseal ossification centers present in different specimens. Flippers from some of the smallest sea-lion pups (CWO-0873, CBH-5, DPD-009) and from the clearly identified fetus (ZC-025, 39.5 cm SL) show ossification only within the diaphyseal centers - i.e. radius, ulna, metacarpals I-V, and in the phalanges (Figs. 1,4). The flipper from one 37-day-old pup (KO-84-1) had eight separate ossifications within the carpal bones. These same carpal ossification centers were significantly larger and more developed in the flippers from three pups-of-the-year (CBH-21, CWO-894, DJS-011), and in one second-year-pup (JBW-061) some fusion of elements had started, whereas more fusion was apparent in one 24-month-old sub-adult (82-M2). It would appear, therefore, that in the thoracic flipper of the California sea lion there is a developmental sequence of appearance and fusion of ossification centers. The radiographs were therefore examined and the presence of new ossification centers was then recorded: these being ossifications within the carpal bones and the epiphyseal ossifications upon the ends of the radius, ulna, metacarpals, and the phalanges. The specimens were then arranged in a sequential order based on the number and size of the carpal and epiphyseal ossification centers detected in the radiographs.

These results are presented in Table 2 and illustrated as radiographs and outline sketches of the radiographs (Figs. 1-9).

In these 13 specimens for which both left and right flippers were radiographed, asymmetrical ossification was only detected in two. In one (CBH-6) the proximal and distal radial epiphyses were detected only in

the left flipper, and in the other one (CWO-875) the second and third carpal bones were ossified only in the left flipper.

Discussion

The radiographic examination of the distal thoracic flipper in the California sea lion has revealed a developmental sequence for the appearance and fusion of specific ossification centers. In the flipper from the smallest sea lion pup - a 39.5 cm fetus - only the diaphyses were ossified, whereas in the flipper from the largest and oldest sea lion - 24-month-old sub-adult - all epiphyseal and carpal centers had ossified and three carpal centers had coalesced into a single bone. Between these two extremes a number of intermediate developmental stages were represented in the series of specimens examined. Unfortunately, however, few of these specimens were of known age.

By the time these sea lions reached one month of age, ten carpal and epiphyseal centers had developed (Table 2, see specimen KO-84-1). The calibration of these specimens to normal full-term newborns is problematic. The standard length (Scheffer, 1967) of newborn Zalophus has been listed as 75 cm (Ronald et al., 1982; King, 1983). More precise data is now available: mean standard length measurements from San Nicolas rookery for normal healthy males at birth was 74.9 ± 3.64 cm (mean ± standard deviation, n=22) and for females was 71.7 ± 4.42 cm (Bonesset al., 1985) This data clearly shows that many of the pups in our study had body lengths below that for normal healthy newborns. One interpretation could be that these dead sea lion pups were in fact born prematurely. Earlier reports have suggested that significant pup mortality may be the result of abortions or premature pupping in California sea lions (see Ronald et al., 1982).

Further interpretation of our radiographic developmental ossification data is hingered by the lack of known-age healthy newborn

pups. The calibration of our sequence requires information on the normal developmental ossification pattern in healthy full-term known-age neonatal sea lion pups.

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Table 1. Collection data for Z. californianus specimens.

Specimen#	R/L[1]	Collection Date	Age[2]	SL[3]	Sex[4]	Site
DPD-004	- +	13 May 1982	Neonate	53.5	F	San Clemente Is.
DPD-005	+ -	13 May 1982	Neonate	61.0	u	San Clemente Is.
DPD-006	+ -	13 May 1982	Neonate	70.0	F	San Clemente Is.
DPD-007	+ -	13 May 1982	Neonate	69.0	F	San Clemente Is.
DPD-008	+ -	13 May 1982	Neonate	59.5	M	San Clemente Is.
DPD-009	+ -	13 May 1982	Neonate	58.5	u	San Clemente Is.
DPD-010	+ -	13 May 1982	Neonate	66.0	F	San Clemente Is.
MSL-219	+ -	26 May 1982	Neonate	74.0	F	San Clemente Is.
MSL-220	+ -	26 May 1982	Neonate	79.0	M	San Clemente Is.
MSL-249	+ -	30 June 1982	Neonate	80.0	F	San Clemente Is.
ZC-026	+ +	14 Feb. 1983	Fetus	39.5	F	Santa Catalina Is.
CBH-1	+ +	16 Apr. 1983	Newborn	59.5	M	San Nicolas Is.
CBH-2	+ -	16 Apr. 1983	Newborn	63.5	M	San Nicolas Is.
CBH-3	+ -	16 Apr. 1983	Newborn	63.5	F	San Nicolas Is.
CBH-4	+ -	16 Apr. 1983	Newborn	u	M	San Nicolas Is.
CBH-5	+ -	16 Apr. 1983	Newborn	59.5	F	San Nicolas Is.
CBH-6	+ +	17 Apr. 1983	Pup	u	u	San Nicolas Is.
CBH-7	+ +	16 Apr. 1983	Pup	61.0	u	San Nicolas Is.
CBH-8	- +	15 May 1983	Pup	76.0	F	San Nicolas Is.
CBH-9	+ -	15 May 1983	Pup	67.0	F	San Nicolas Is.
CBH-10	+ -	15 May 1983	Pup	70.0	F	San Nicolas Is.
CBH-11	+ -	15 May 1983	Pup	62.0	F	San Nicolas Is.
CBH-12	+ -	15 May 1983	Pup	67.0	F	San Nicolas Is.
CBH-13	+ -	15 May 1983	Pup	76.0	M	San Nicolas Is.
CBH-14	+ -	15 May 1983	Pup	70.0	F	San Nicolas Is.
CBH-15	+ -	15 May 1983	Pup	81.0	M	San Nicolas Is.
CBH-19	+ -	5 June 1983	Pup	u	u	San Nicolas Is.
CBH-21	+ -	22 Oct. 1983	Pup of Year	80.0	F	San Nicolas Is.
CWO-0873	+ +	30 Mar. 1983	Prem. Pup	u	u	San Clemente Is.
CWO- 875	+ +	16 Apr. 1983	Neonate	u	F	San Clemente Is.
CWO-0876	+ +	16 Apr 1983	Neonate	u	u	San Clemente Is
CWO- 886	+ +	13 May 1983	Neonate	u	M	San Clemente Is.
CWO-0894	+ +	5 Nov. 1983	Pup of Year	82.0	F	San Clemente Is.
LMF-0001	+ +	14 May 1983	Neonate	71.5	F	San Clemente Is.
DJS-011	+ +	19 Jan 1983	Pup of Year	91.0	M	San Nicolas Is.
KO-84-1	+ +	29 July 1984	37 Day	-	F	San Nicolas Is.
JBW-062	+ +	20 Sept.1984	2nd Year	82.0	F	San Clemente Is.
82-M2	+ -	9 Jul. 1982	24 Month	-	F	Captive

[1] Right and/or left flipper collected.

[2] Age category as recorded by field collector. A short (2-5 cm) and moist umbilical cord was present on four "newborns" (CBH-1, CBH-3, CBH-4, CBH-5) and in addition one of these was still partially covered by its amniotic sac (CBH-4).

[3] Standard length (SL) was measured and recorded in centimeters as recommended by Scheffer (1967).

[4] u - unknown.

Table 2. Appearance (+) and Fusion (f) of ossification centers in the Distal Thoracic Flipper of *Zalophus californianus*.

Specimen #	Sex	SL (cm)	CARPALS: Radiale	Intermedium	Centrale	Ulnare	Accessory	First	Second	Third	Fourth	EPIPHYSES: Proximal radial	Distal radial	Proximal ulnar	Distal ulnar	Proximal Metacarpal I	Distal " "	" " II	" " III	" " IV	Proximal Phalanx 1, I	" " II	" " III	" " IV	Proximal Phalanx 2, II	" " III	" " IV	Radius Length (mm)	Number of Centers
ZC-026	F	39.5																										25.0	0
CWO-0873	u	u																										30.0	0
CBH-5	F	59.5																										46.0	0
DPD-009	u	58.5																										49.5	0
CWO-0876	u	u						++																				47.5	2
CBH-7	u	61.0								+		++																42.5	3
DPD-008	M	59.5	+					++																				49.5	3
CBH-6	u	u						++				++																43.0	4
CBH-1	M	59.5	++					+				+																48.0	4
CBH-4	M	u	++					++	+			+																43.0	6
CWO-875	F	u	++					++++																				54.0	6
CBH-2	M	63.5	++					++++				++																45.5	8
CWO-886	M	u	++	+				++++																				48.0	8
DPD-004	F	53.5	+++					++++				++																45.5	9
CBH-9	F	67.0	++	+				++++				++																47.0	9
LMF-0001	F	71.5	++	+				++++				++																49.5	9
DPD-005	u	61.0	+++					++++				++																50.5	9
CBH-12	F	67.0	++	+				++++				++																50.5	9
CBH-10	F	70.0	++	+				++++				++																50.5	9
MSL-219	F	74.0	++	+				++++				++																51.5	9
MSL-220	M	79.0	++++					++++				+																54.0	9
CBH-3	F	63.5	++++					++++				++																44.5	10
CBH-15	M	81.0	++++					++++				++																52.5	10
KO-84-1	F	u	++++					++++				++																67.0	10
DPD-010	F	66.0	++++					++++				++				+												51.0	11
DPD-006	F	70.0	++	+				++++				++				+					+							53.0	11
CBH-19	u	u	++++					++++				+				+					+							66.0	11
CBH-8	F	76.0	++++					++++				++				+					+							49.0	12
CBH-14	F	70.0	++++					++++				++				+					+							49.5	12
CBH-13	F	67.0	++++					++++				++				+					+							51.0	12
DPD-007	F	69.0	++++					++++				++				+					+							52.5	12
MSL-249	F	80.0	++++					++++				++				+					+							58.0	12
CBH-11	F	62.0	++++					++++				++				++++					+							53.5	15
CBH-21	F	80.0	+++++					++++				++		+		+++++					+++++				+			66.0	23
CWO-0894	F	82.0	++++					++++				++		+		+++++					+++++				+++			70.0	24
DJS-011	M	91.0	++++					++++				++		+		+++++					+++++				+++			77.0	24
JBW-062	F	82.0	fff	++				++++				++		+		+++++					+++++				+++			81.5	25
82-M2	F	u	fff	++				++++				++		++		+++++					+++++				+++			107.0	26

Fig 1. Outline sketches of the ossification centers in the carpal region of 6 California sea lion pups (life size, drawn from the radiographs). Specimens DPD-009, DPD-008, DPD-005, DPD,004, MSL-220, and DPD-006; arranged in an approximate developmental sequence.

Key: a = accessory carpal bone
c = centrale carpal bone
e = epiphyseal ossification center
i = intermedium carpal bone
r = radiale carpal bone
u = ulnare capal bone
1 = first carpal bone
2 = second carpal bone
3 = third carpal bone
4 = fourth carpal bone
I = first metacarpal bone
II = second metacarpal bone
III = third metacarpal bone
IV = fourth metacarpal bone
V = fifth metacarpal bone

Note: The flippers in all figures - outline sketches and radiographs- have been illustrated in a constant format. Whether left or right, each flipper is illustrated so that the radius and the first digit are always on the left side of the image.

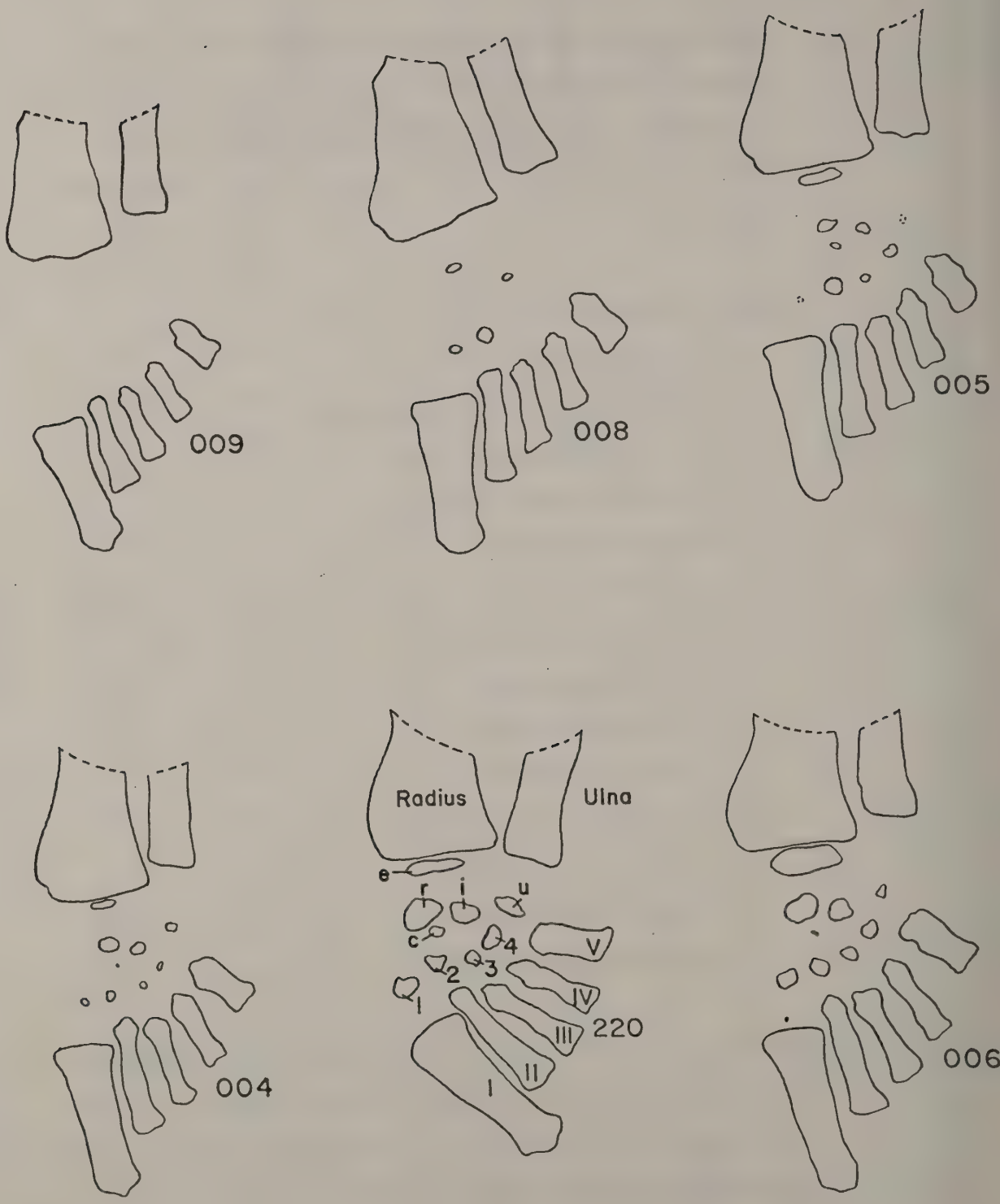

Fig. 1.

Fig. 2. Outline sketches of the ossification centers in the carpal region of 3 California sea lion pups (life size, drawn from the radiographs). Specimens DPD-007, DPD-010, and MSL-249; arranged in an appropriate developmental sequence.

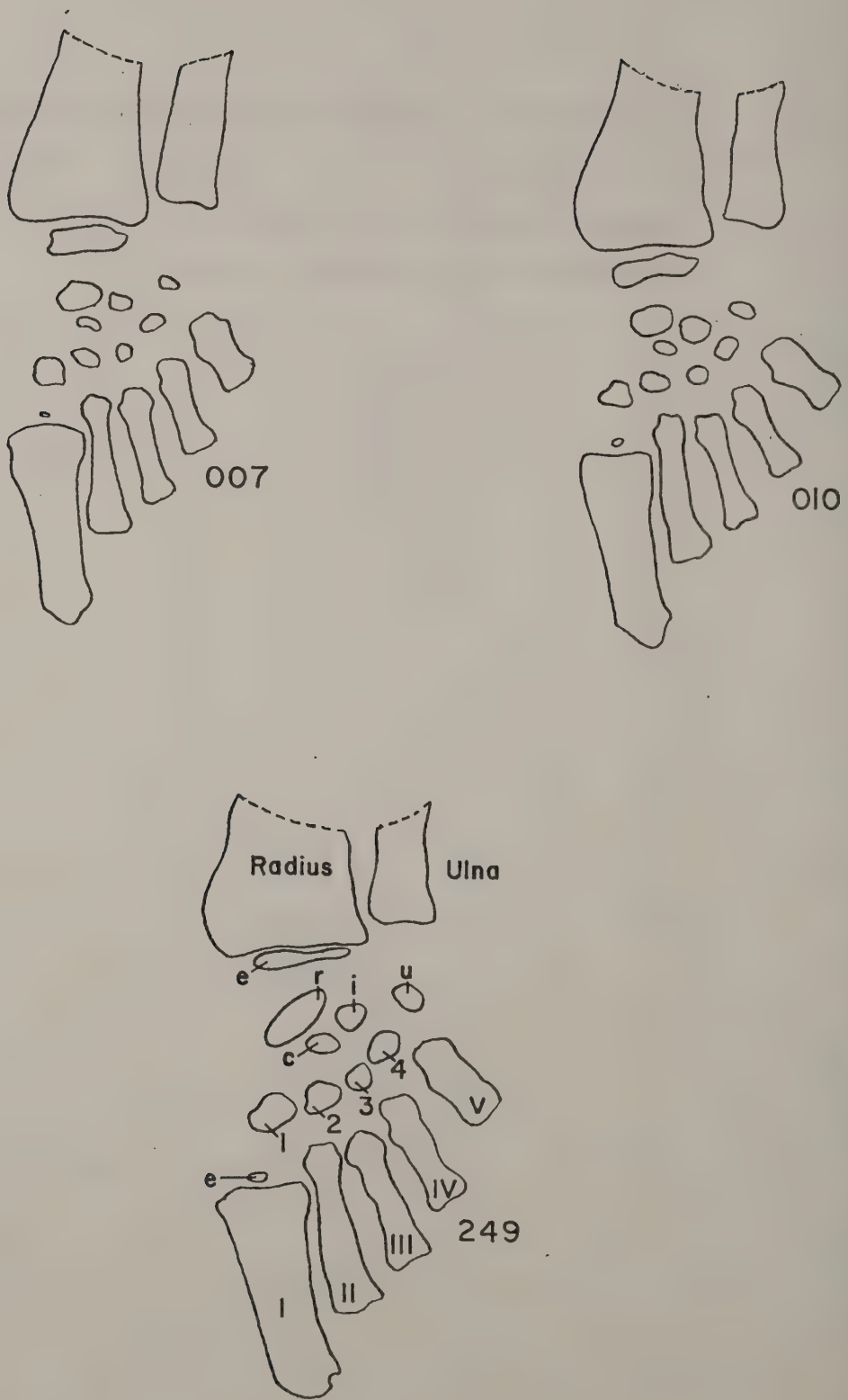

Fig. 2.

Fig. 3. Outline sketch of the ossification centers in the carpal region of a 24-month-old female California sea lion (life size, drawn from the radiograph). Specimen 82-M2.

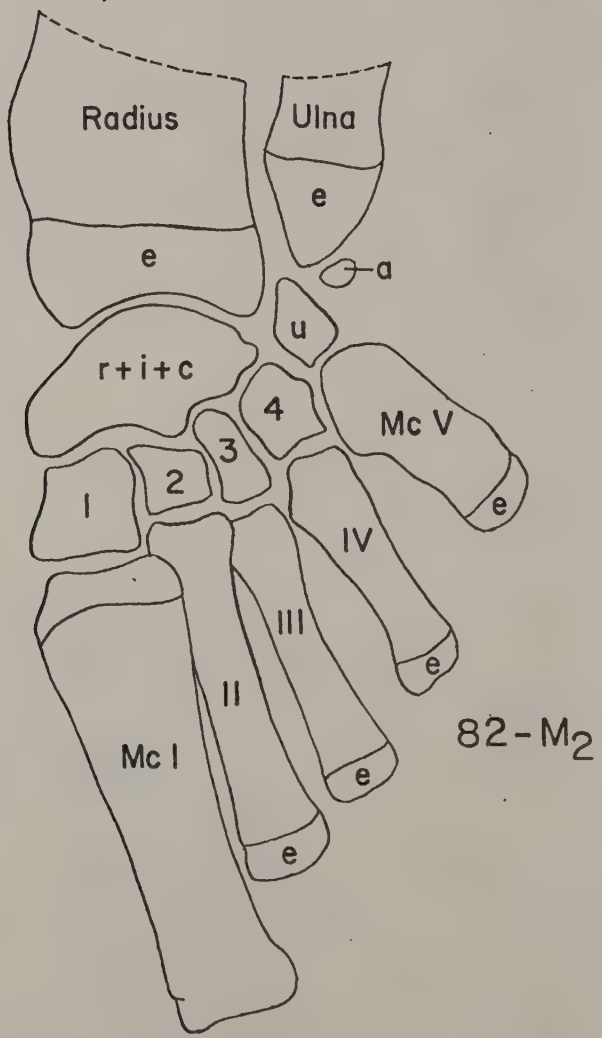

Fig. 3.

Fig. 4a, 4b. Radiographs of the thoracic flipper from 58.8 cm California sea lion pup showing the earliest developmental stage. The carpal bones and epiphyses are not ossified. Specimen DPD-009.

a. Distal flipper.

b. Carpal region.

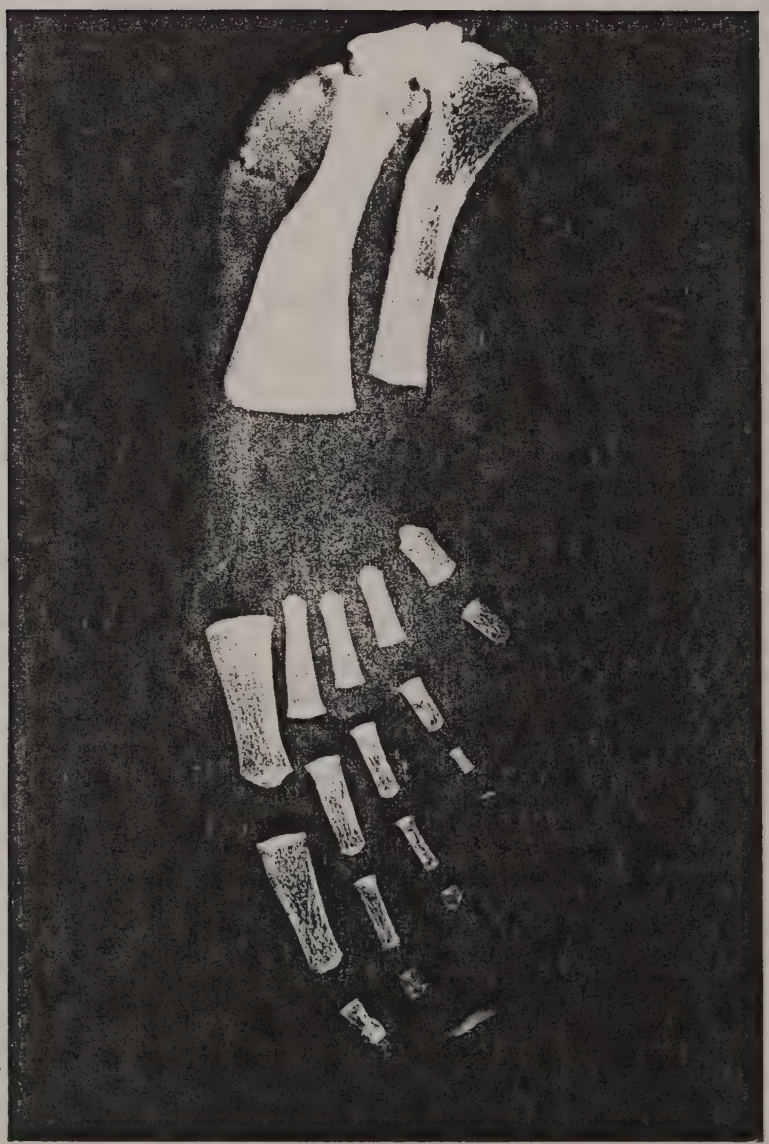

Fig. 4a

Fig. 4b

Fig 5a, 5b. Radiographs of the thoracic flipper from a California sea lion neonatal pup showing an early developmental stage. Ossification has commenced in the radiale, intermedium, and in all four distal carpal bones. Specimen CWO-875.

a. Distal flipper.

b. Carpal region.

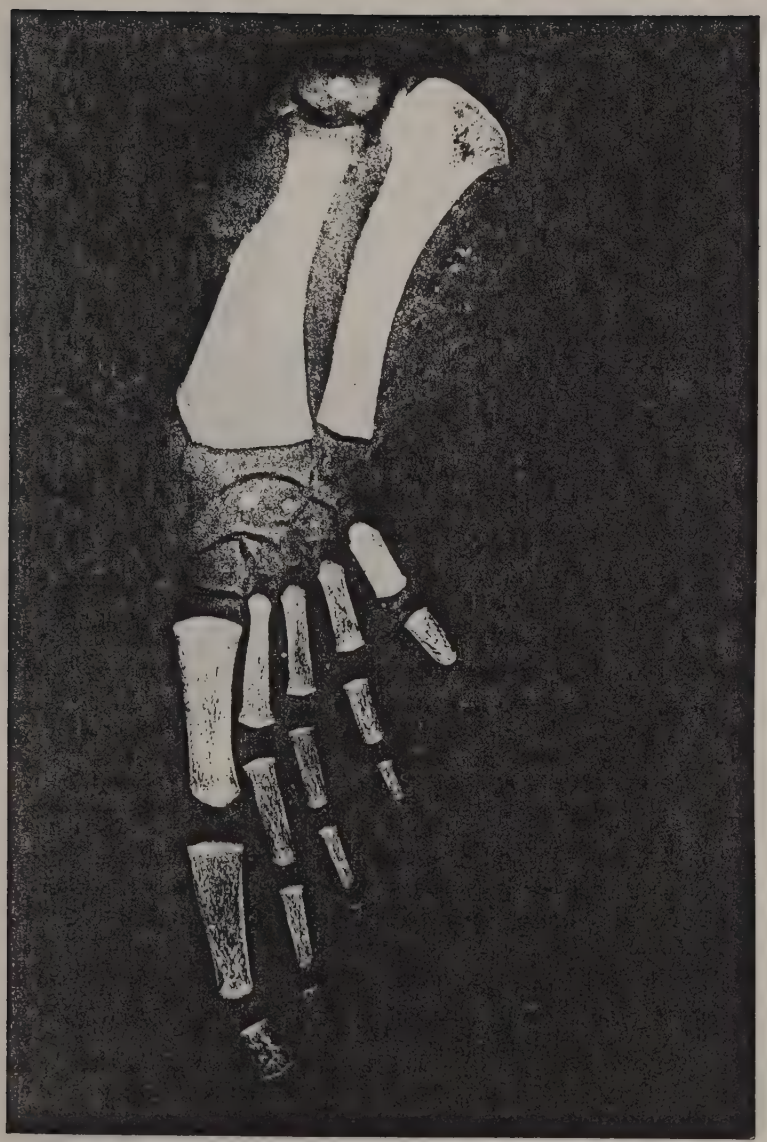

Fig. 5a

Fig. 5b

Fig. 6a, 6b. Radiographs of the thoracic flipper from a 70.0 cm California sea lion neonatal pup. Compared to Figure 5, the carpal ossification centers are more developed: the ulnare and the distal radial epiphyseal center have now ossified. Specimen CBH-10.

a. Distal flipper.

b. Carpal region.

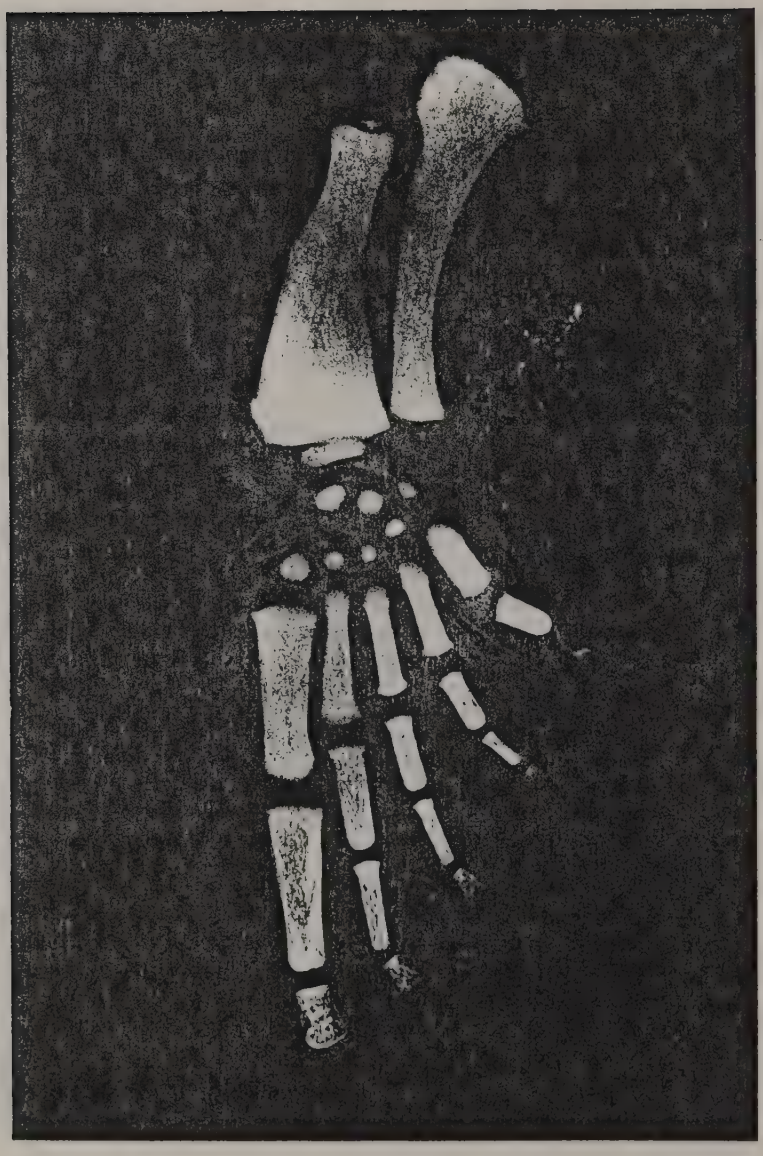

Fig. 6a

Fig. 6b

Fig 7a, 7b. Radiographs of the thoracic flipper from a 79.0 cm California sea lion neonatal pup. Compared to Figure 6, the carpal ossifications are now larger and the centrale is now ossified. Specimen MSL-220.

a. Distal flipper.

b. Carpal region.

Fig. 7a

Fig. 7b

Fig 8a, 8b. Radiographs of the thoracic flipper from a 91.0 cm pup-of-the-year California sea lion, approximately 7-months-old. Carpal ossifications are very well developed although no fusion is apparent. Specimen DJS-011.

a. Distal flipper.

b. Carpal region.

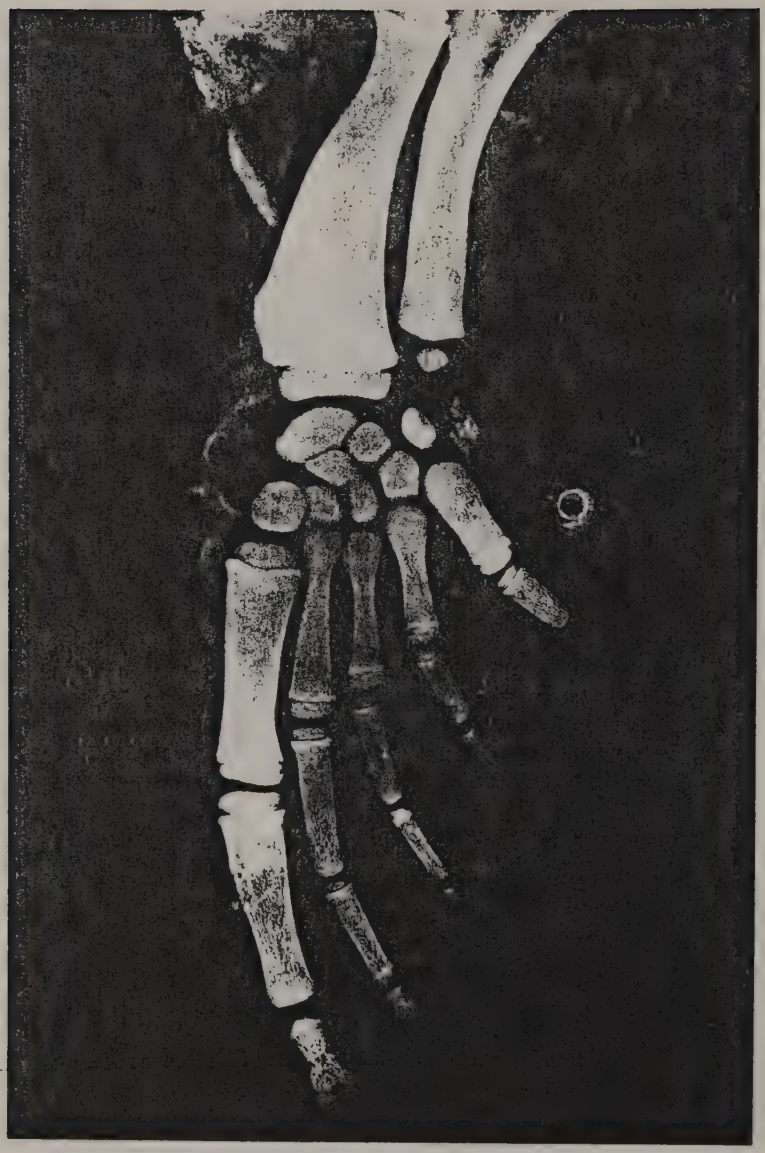

Fig. 8a

Fig. 8b

Fig. 9a

Fig. 9a, 9b. Radiographs of the thoracic flipper from a sub-adult 24-month-old female California sea lion. The radiale, intermedium, and centrale have fused to form the radial carpal bone, and the accessory carpal bone has now ossified. Specimen 82-M2.

a. Distal flipper.

b. Carpal region.

Fig. 9b